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Author(s)	Kobayashi, Naomasa
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COMPARATIVE TOXICITY OF VARIOUS CHEMICALS, OIL EXTRACTS AND OIL DISPERSANT EXTRACTS TO CANADIAN[1] AND JAPANESE[2] SEA URCHIN EGGS[3]

Naomasa KOBAYASHI

Biological Laboratory, Doshisha University, Kyoto

With Tables 1–4

Introduction

Techniques used to measure and judge the effects of marine pollution are many and varied. To the marine biologist it is most desirable to learn how pollutants may influence living organisms. In some instances the presence of pollutants, occurring at concentrations below the level of detection by chemical analysis, may be detected through biological assays. Bioassays also may identify the presence of deleterious substances—as a first step in the chemical identification of pollution. Such bioassays are most revealing when a sensitive organism or biological system is used as the detector. Eggs and embryonic stages of marine organisms in general tend to be sensitive detectors of pollution.

Earlier the author proposed the use of sea urchin eggs and embryos as general indicator organisms in marine pollution bioassays (Kobayashi, 1971); sensitivity of the methods employed subsequently was increased by the use of "aged" eggs (Kobayashi, 1974). Later it was recognized that pollution effects on the formation of the pluteus were more pronounced than in any of the earlier stages (Kobayashi, 1977). These earlier studies used eggs mainly of the Japanese sea urchin *Anthocidaris crassispina*.

This paper examines eggs and embryonic responces to the substances used earlier, namely the sulphates or chlorides of Cu, Zn, Cd and ammonia, as well as alkyl benzyl sulphonate (ABS); water soluble extracts of Bunker C oil and of the oil dispersant BP 1100X, and of oil-oil dispersant mixtures. Comparisons are made of the sensitivity of eggs from three different sea urchin species.

Materials and Methods

Eggs of *S. droebachiensis* were obtained and used at the Pacific Biological Station,

Nanaimo, B.C. Eggs of the Japanese species (*H. pulcherrimus, A. crassispina*) were used primarily at the Seto Marine Biological Laboratory, Wakayama Prefecture, Japan. In all instances the eggs were fertilized in the test solutions. Rates of fertilization, developmental success to first cleavage, gastrulation, pluteus formation, and as occurrence of developmental anomalies were determined. The test temperatures used (15, 18, 28°C) are somewhat higher than those at which *S. droebachiensis* (9°C), *H. pulcherriums* (14°C) and *A. crassispina* (24°C) are found in nature. There were no significant developmental anomalies occur at these temperatures. Eggs were obtained using the KCl method (0.5 M potassium chloride solution injected into the body cavity). The eggs were shed into sea water, rinsed several times with fresh sea water and fertilized within 1 hour. Sperm were used within 1 hour of removal of the testes, at a dilution (V/V) of 1:1000 with sea water.

Following fertilization, the percentage was obtained of eggs that initially showed an elevated fertilization membrane. First cleavage occurred about 120, 90 and 60 min. after insemination at 15, 18 and 28°C, respectively. At those times the rate and state of cleavege was determined, providing estimates of the proportion of undivided cells, normal two-cell divisions, and multicell states associated with polyspermy. Secondly the number of reduced blastulae, normal gastrulae, and exogastrulae among the free swimming embryos was recorded about 48 h. after fertilization at 15°C, 24 h. at 18°C and 12 h. at 28°C. Exogastrulae are those gastrula stages in which the rudiment of the digestive organ, normally formed inside the embryo by invagination, develops outside the embryo. Finally, the number of normal and abnormal free-swimming plutei was recorded 3 days after fertilization at 15°C, 2 days at 18°C and 1 day at 28°C. Abnormal plutei included those in which normal "skeletal" elements failed to form. At each examination, 100 eggs or embryos were examined in each three full replicates of the test conditions.

Series of concentrations of the chemicals to be tested were prepared by successive dilution of the original solution (chemicals—1:10⁴–10³ sea-water in volume=10,000–1,000 ppm). The oil or oil dispersant seawater extracts were prepared by mixing Bunker C oil or BP 1100X oil dispersant and normal sea water (1:3 or 1:10 in volume= 330,000–100,000 ppm) in a beaker by magnetic stirrer for 30 min. This provided a water soluble fraction in sea water as required.

Results

The effects of Cu, Zn, Cd, ammonia and ABS on fertilization and development of eggs of *S. droebachiensis* are summarized in Table 1. In general, the effects noted compare with those obtained for other species (Kobayashi 1971, 1974, 1977) although there are suggested differences in species sensitivity.

Heavy metals

In the highest concentrations of Cu, most eggs remained unfertilized. Two hours after insemination, reduced development was evident in comparison with

Table 1. Effects of Cu, Zn, Cd, ABS and ammonia on egg development in *Strongylocentrotus drœbachiensis*. Tests: March–April 1978; water temperature: 15°C.

Chemical	Conc.	Time after insemination							
		3 min.	2 h.			2 days			3 days
	ppm	fertil. memb. formation (%)	1-cell state (%)	2-cell stage (%)	multicell polyspermy (%)	arrested blastula (%)	normal gastrula (%)	exo-gastrula (%)	pluteus stage
CuSO$_4$·5H$_2$O	1.0	8	93	7	0	—	—	—	unfertilized
	0.5	19	82	13	5	—	—	—	cytolysis
	0.2	76	25	71	4	—	—	—	cytolysis
	0.1	88	15	84	1	—	—	—	cytolysis
	0.05	95	7	93	0	32	68	0	retardation
	0.02	98	3	96	1	7	93	0	normal
	Control	97(>90)	4	96(>90)	0	5	95(>90)	0	normal
ZnCl$_2$	1.0	2	98	2	0	—	—	—	unfertilized
	0.5	21	81	17	2	100	0	0	cytolysis
	0.2	35	65	32	3	98	0	2	cytolysis
	0.1	67	34	64	2	97	3	0	arrested blast.
	0.05	91	10	89	1	2	98	0	retardation
	0.02	92	7	93	0	2	98	0	normal
	Control	92(>90)	9	91(>90)	0	3	97(>90)	0	normal
CdCl$_2$·2½H$_2$O	20	78	53	45	2	—	—	—	4–8 cell
	10	91	11	88	1	—	—	—	morula
	5	93	8	91	1	100	0	0	retardation
	2	95	6	93	1	100	0	0	retardation
	1	97	5	95	0	31	69	0	retardation
	0.5	96	6	94	0	9	91	0	normal
	Control	97(>90)	7	91(>90)	2	5	95(>90)	0	normal
ABS	20	53	52	48	0	—	—	—	cytolysis
	10	64	58	42	0	88	12	0	cytolysis
	5	93	9	91	0	31	69	0	retardation
	2	91	12	88	0	9	91	0	no skeleton
	1	94	8	92	0	7	93	0	normal
	Control	97(>90)	5	95(>90)	0	5	95(>90)	0	normal
NH$_4$Cl	50	59	52	46	2	—	—	—	cytolysis
	20	92	9	90	1	—	—	—	cytolysis
	10	89	12	87	1	—	—	—	cytolysis
	5	93	8	92	0	100	0	0	cytolysis
	2	94	7	93	0	15	85	0	almost normal
	1	92	9	90	1	5	95	0	normal
	Control	92(>90)	9	91(>90)	0	3	97(>90)	0	normal

cell division in the controls. Polyspermy occurred in most concentrations of Cu. Two days after fertilization the limited data suggest some evidence of arrested blastulae. Three days after fertilization, in the pluteus stage, response to copper extended to lower concentrations: those at 0.02 ppm were normal, retarded development was evident at 0.05 ppm, and cytolysis had occurred at higher concentrations.

Response to Zn essentially was similar to that for copper. Fertilization membrane formation and subsequent cell division were reduced in higher concentrations of zinc, there was evidence of polyspermy, and reduced development occurred at the blastula stage two days after fertilization. Pluteus formation was normal in 0.02 ppm Zn, with progressively greater retardation and cytolysis at higher concentrations.

Cadmium had less influence on fertilization membrane formation than copper or zinc. However, development was reduced in higher Cd concentrations, and there was evidence of formation of reduced blastula at intermediate concentrations. At the pluteal stage, development was normal only at 0.5 ppm; retardation occurred was evident at all higher concentrations.

Other chemicals

Higher concentrations of ABS reduced fertilization membrane formation, and reduced development was evident in the initial stages of cell division. There was no evidence of polyspermy. However, extensive numbers of arrested blastulae occurred in intermediate concentrations. On the 3rd day, pluteal development was normal in 1 ppm ABS; development was anomalous or retarded, extending to cytolysis in higher concentrations. Lack of internal skeletal formation also was observed in gastrulae in 2 ppm ABS.

Quantitatively, the influence of ammonia was similar to that of ABS. Some reduced fertilization membrane formation occurred at the highest concentrations, which continued to reduced initial cell division. There was some evidence of polyspermy. However, development of the normal pluteus stage was limited to concentrations lower than 2 ppm.

In summary, at the pluteal stage the relative toxicity of the five compounds tested was $Cu > Zn > Cd > ABS > NH_3$.

Oil and oil dispersant

Concentrations of Bunker C oil and BP 1100X oil dispersant water extracts used, their mixtures, and associated responses in sea urchin eggs are summarized in Table Table 2 a-c.

Bunker C oil extracts. In *S. droebachiensis* eggs, fertilization did not occur in the highest concentration of the Bunker C oil extract (Table 2a). Intermediate concentrations reduced fertilization membrane formation and subsequent cleavage; there also was evidence of polyspermy and formation of reduced blastulae. Pluteus formation was retarded at concentrations greater than 5 ppm. In *H. pulcherrimus*, fertilization membrane formation and cleavage were reduced at intermediate and higher con-

Table 2. Effects of water extracts of Bunker C oil, BP 1100X oil dispersant, and mixtures of the two on egg development in three species of sea urchin.

(a) *Strongylocentrotus droebachiensis,* March 1978; water temperature, 15°C.

Chemical	Conc. ppm	3 min. fert. memb. formation (%)	2 h. 1-cell state (%)	2-cell stage (%)	multicell polyspermy (%)	2 days arrested blastula (%)	normal gastrula (%)	exo-gastrula (%)	3 days pluteus stage
Bunker C oil	50	0	100	0	0	—	—	—	unfertilized
1:3 sea water	20	63	38	59	3	43	57	0	retardation
extract	10	87	25	73	2	11	89	0	somewhat ret.
	5	92	8	88	4	7	93	0	normal?
Oil dispersant	200,000	57	54	46	0	—	—	—	cytolysis
BP 1100X	100,000	89	13	87	0	12	88	0	retardation
1:10 sea water	50,000	96	4	96	0	8	92	0	somewhat ret.
extract	20,000	93	8	92	0	6	94	0	normal
Mixture	20+100,000	58	47	51	2	69	31	0	no skeleton
Oil+dispersant	10+50,000	83	25	74	1	12	88	0	abnormal
extracts	5+20,000	91	7	87	6	7	93	0	retardation
	2+10,000	97	5	91	4	8	92	0	normal?
Control		97(>90)	5	94(>90)	1	9	90(>90)	1	normal

(b) *Hemicentrotus pulcherrimus,* January 1978; water temperature, 18°C.

Chemical	Conc. ppm	3 min. fert. memb. formation (%)	90 min. 1-cell state (%)	2-cell stage (%)	multicell polyspermy (%)	1 day arrested blastula (%)	normal gastrula (%)	exo-gastrula (%)	2 days pluteus stage
Bunker C oil	100	35	71	29	0	—	—	—	2-4 cells
1:3 sea water	50	66	45	55	0	81	19	0	no skeleton
extract	20	82	24	76	0	53	47	0	retardation
	10	97	5	95	0	9	91	0	somewhat ret.
	5	96	0	100	0	2	98	0	normal
Oil dispersant	200,000	22	100	0	0	—	—	—	unfertilized
BP 1100X	100,000	55	100	0	0	—	—	—	no cleavage
1:10 sea water	50,000	72	38	62	0	81	19	0	somewhat ret.
extract	20,000	98	5	95	0	2	98	0	normal
Control		98(>90)	3	97(>90)	0	2	98(>90)	0	normal

(c)   *Anthocidaris crassispina,* September 1978; water temperature, 28°C.

Chemical	Conc.	Time after insemination							
		3 min.	1 h.			12 h.			1 day
	ppm	fert. memb. formation (%)	1-cell state (%)	2-cell stage (%)	multicell polyspermy (%)	arrested blastula (%)	normal gastrula (%)	exo-gastrula (%)	pluteus stage
Bunker C oil	100	42	63	37	0	—	—	—	blastula
1:3 sea water	50	71	32	68	0	67	33	0	retardation
extract	20	84	19	81	0	30	70	0	somewhat ret.
	10	95	7	93	0	1	99	0	normal
Oil dispersant	200,000	26	97	3	0	—	—	—	unfertilized
BP 1100X	100,000	63	72	28	0	—	—	—	cytolysis
1:10 sea water	50,000	81	46	54	0	83	17	0	retardation
extract	20,000	98	5	95	0	3	97	0	normal
Mixture Oil +dispersant extracts	50+100,000	34	82	18	0	—	—	—	cytolysis
	20+50,000	68	49	51	0	86	14	0	retardation
	10+20,000	87	15	85	0	17	83	0	retardation
	5+10,000	92	10	90	0	5	95	0	normal
Control		98(>90)	3	97(>90)	0	2	98(>90)	0	normal

centrations (Table 2b). There was no evidence of polyspermy, but arrested blastulae occurred at intermediate and higher concentrations. Pluteus formation was retarded or abnormal at concentrations greater than 5 ppm. Lack of internal skeletal formation also was observed in gastrulae. In *A. crassispina,* effects of the oil extract (Table 2a) were similar to those for *H. pulcherrimus.* Retarded or abnormal pluteus formation occurred at concentrations greater than 10 ppm. In general, sensitivity of the three species to the oil extract was in the order *S. droebachiensis* > *H. pulcherrimus* > *A. crassispina.*

Oil-oil dispersant extract. For the three species of sea urchin (Tables 2a-c), reduced fertilization membrane formation and reduced cleavage occurred, increasing in frequency in the higher concentrations. There was no evidence of polyspermy. *H. pulcherrimus* appeared more sensitive than the other species; cleavage failed to occur at all in eggs at the higher concentrations. For the three species, pluteus formation was normal in 20,000 ppm; at higher concentrations it was either reduced or abnormal. Although response of the eggs of the three species to the dispersant was similar, sensitivity appeared to be in the order *H. pulcherrimus* > *A. crassispina* > *S. droebachiensis.*

Oil-oil dispersant mixture extracts. Comparison of the effects of the mixture extract can be made for eggs of *S. droebachiensis* and *A. crassispina* (Tables 2a, 2c). Such tests were not conducted on eggs of *H. pulcherrimus.* Tested alone, 5 ppm of oil extract or 20,000 ppm of dispersant extract did not prevent normal pluteus forma-

tion in eggs of *S. droebachensis*. However, an extract from a mixture of these two concentrations retarded pluteus formation, and pluteus formation was normal in the extract of a mixture of 2 ppm of oil and 10,000 ppm of dispersant. By comparison pluteus formation in *A. crassispina* eggs was normal in individual tests of extracts of 10 ppm of oil, and of 20,000 ppm of dispersant, when tested alone. In combination, the mixture extract at these concentrations retarded pluteus formation. At lower concentrations, pluteus formation was normal in mixture extracts of 5 ppm of oil and 10,000 ppm of dispersant. Hence, it appears that extracts of oil-oil dispersant mixtures are more toxic to the eggs of both species examined than are the components alone.

The effects of the oil, dispersants, and oil-dispersant mixtures on egg development in the three species are summarized as follows. The Canadian species (*S. droebachiensis*) is more sensitive to Bunker C oil than the Japanese species (*H. pulcherrimus, A. crassispina*). On the other hand, the two Japanese species are more sensitive to the oil dispersant than the Canadian species. The extract of mixtures of oil and dispersant, however, suggest that *S. droebachiensis* and *A. crassispina* are about equal in sensitivity. For these two species the toxicity of the oil-oil dispersant mixture was greater than that of the components tested alone. This is true particularly for the Japanese species.

Discussion

Heavy metals and other chemicals

Numerous accounts of the biological effects of single toxicants abound in the literature. Waterman (1937), Okubo and Okubo (1962), Kobayashi (1971, 1974 and 1977) and Murakami *et al.* (1976) conducted comprehensive tests with series of metallic salts. Their reports show the relative toxicity of three important salts to be in the order $Cu > Zn > Cd$; similar results were obtained in the current experiments. In general, the effect noted on sea urchin egg development is one of retardation and inhibition of cell division. These initial effects may be followed by

Table 3. Estimated threshold concentrations associated with arrested cleavage in sea urchin eggs (Heavy metals, ABS, NH₃).

Species	Estimated threshold concentrations (ppm)					
	Cu	Zn	Cd	ABS	NH₃	Source
Strongylocentrotus	0.02	0.02	0.5	1.0	1.0	This paper
Pseudocentrotus	0.03	0.03	1.0	1.0	2.0	This paper
Hemicentrotus	0.05	0.05	2.0	1.0	1.0	This paper
	0.01	—	—	—	3.2	Okubo and Okubo (1962)
	—	—	—	1.25	—	Sawada and Ohtsu (1975b)
	0.006	0.07	0.1	—	—	Murakami *et al.* (1976)
Anthocidaris	0.06	0.06	1.5	1.5	3.0	This paper
	0.03	0.03	—	—	3.2	Okubo and Okubo (1962)
Arbacia	0.001	0.002	0.5	—	—	Waterman (1937)

arrested development, abnormal differentiation and destruction of the embryo.

Estimates of threshold concentrations*, above which problems associated with cleavage are seen, are summarized in Table 3. Data from other sources also are included, including unpublished data of the author for *Pseudocentrotus depressus*. Undoubtedly some of the differences in tabled values are a result of using different sets of experimental conditions. With that proviso, some general comments can be made. *Arbacia* may be more sensitive to Cu and Zn than are the other species tabled. With the exception of *Arbacia*, sensitivity in the remaining species appears similar, but possibly in the order *Strongylocentrotus* > *Pseudocentrotus* > *Hemicentrotus* > *Anthocidaris*. Of the five materials compared, toxicity appears to be in the order Cu > Zn > Cd > ABS > NH₃.

Oil and oil dispersant

The sensitivity of marine eggs and larvae depends to a considerable extent on the species and stages examined (Craddock, 1977; Johnson, 1977; Kühnhold, 1977; Rice *et al*, 1977). The eggs and larvae of echinoderms are known to be very sensitive to petroleum products. Watersoluble extracts of crude or refined oils (at concentration up to 1,000 ppm) appear to be less toxic to sea urchin eggs at fertilization or during early embryogenesis in comparison with later developmental stages (Allen, 1971; Chia, 1973; Rensoni, 1974; Straughan, 1976; Lönning and Hagström, 1975a, 1976; Lönning, 1977; Nichol *et al.*, 1977). Exceptions also occur (Sawada and Ohtsu, 1975a). Lighter oil products are less toxic than heavier crude or bunker oils (Allen, 1971; Sawada and Ohtsu, 1975a; cf. Nichol *et al.*, 1977). In the current study, Bunker C oil was very toxic to sea urchin eggs (threshold concentrations, 5–10 ppm; Table 4); in comparison, threshold values for other heavy oils have been found by the author (unpublished data) to be higher (1,000 ppm, *Hemicentrotus*; 3,000 ppm, *Anthocidaris*).

In general, the effects of oil-dispersant chemicals appear to be moderate in early stages of fertilization and cleavage. Harmful effects are more evident in later embryonic stages (gastrula, pluteus). In Japan, many types of oil dispersant chemicals are available. Many of the newer compounds are less toxic than those developed earlier. For example, threshold concentrations of some dispersants to embryonic stages of *H. pulcherrimus* are in the range of 3.2–320 ppm (Okubo *et al.*, 1972), 0.32–3.2 ppm (Honma and Kitami, 1974), 2–20 ppm (Sawada and Ohtsu, 1975a)

Table 4. Estimated threshold concentrations associated with delayed development in sea urchin eggs (Bunker C oil, BP 1100X oil dispersant extracts, and mixtures of extracts).

Species	Bunker C oil (ppm)	BP 1100X dispersant (ppm)	Oil-dispersant mixtures (ppm)
Strongylocentrotus	5.0	20,000	2.0+10,000
Hemicentrotus	5.0	20,000	—
Anthocidaris	10.0	20,000	5.0+10 000

* Those concentrations above which a response ceases to be zero under a given set of conditions.

and 1–10 ppm (Kobayashi, unpublished). Among dispersants reported elsewhere, Corexit 9527 has been tested on several species of sea urchin. A concentration of 1–10 ppm caused problems in fertilization and development (*Paracentrotus lividus, Echinocyamus pusillus, S. droebachiensis* and *S. pallidus*), often resulting in formation of pathological larvae or rapid cytolysis (Lönning and Hagström, 1976). More moderate effects were observed with Corexit 8666, at 2000 ppm (Lönning and Hagström, 1975a), and with Corexit 8666, Corexit 7664, BP 1100 and BP 1100X at 1000 ppm, using *S. droebachiensis* and *S. pallidus* (Lönning and Hagström, 1975b) and with BP 1100, BP 1100X and BP 1100WD at concentrations of 1000, 1000 and 100 ppm, respectively, using *S. pallidus* eggs (Lönning, 1977).

Mixtures of oil and dispersants, in general, are more toxic to sea urchin eggs than the components presented alone. Sawada and Ohtsu (1975a) found this to be true with some mixtures. Lönning and Hagström (1975a) found a mixture of crude oil and Corexit 8666 to act in this manner, as did oil and Corexit 9527 (Lönning and Hagström, 1976). Similar results have been obtained by the author (unpublished, *H. pulcherrimus* eegs) in addition to these reported here.

Threshold concentrations for Bunker C oil and BP 1100X dispersant are similar for the three species reported (Table 4), although *A. crassispina* may be somewhat less sensitive. Effective concentrations for inhibition of cleavage at the 2-cell stage suggest that the response to Bunker C oil is more pronounced in *S. droebachiensis* than in *H. pulcherrimus* or *A. crassispina*. The opposite appears to be true for response to BP 1100X (Table 4). Effects of extracts of mixtures of the oil and dispersant appear similar for the two Japanese species (Table 4). The results suggest that maximum protection of marine ecosystems would require removal of spilled oil (by pumping or absorption) rather than by its dispersal. Pronounced ecological effects likely would be found in shallow seas where productivity is high and the potential for dilution of dispersed oil may be limited.

In summary, threshold concentrations of the various toxicants tested are rather similar for the five species of sea urchin examined (Table 4). Some of the differences noted may be true differences in species sensitivity, others likely are associated with differences in test conditions. There appears to be a small trend in sensitivity such that *Arbacia* > *S. droebachiensis* > *P. depressus* > *H. pulcherrimus* > *A. crassispina*. For these species, the toxicity of the various materials examined are approximately $Cu >$ $Zn > Cd > ABS > NH_3 >$ Bunker C oil > BP 1100X oil dispersant. The bioassay method itself, using sea urchin eggs, is considered a useful one for its simplicity, sensitivity and uniformity. Sea urchins are available in many marine environments. Their similarity in sensitivity to various pollutants would make them a useful candidate organism for standard marine pollution tests throughout the world.

Summary

The effects of various toxic agents were examined in eggs and early embryonic stages of a sea urchin from the Pacific coast of Canada (*Strongylocentrotus droebachiensis*)

and compared with those obtained for two species from Japanese waters (*Hemicentrotus pulcherrimus*, *Anthocidaris crassispina*).   Toxic agents included sulphates or chlorides of Cu, Zn, Cd and NH_3; alkyl benzyl sulphonate (ABS); and water-soluble extracts of Bunker C oil and of BP 1100X oil dispersant.   Responses observed included departures from control rates of fertilization, cleavage and gastrulation.   Developmental anomalies were noted at the pluteus stage, reduced development at the blastula stage, and exogastrulae.   Heavy metals induced polyspermy and produced reduced blastulae and exogastrulae.   ABS and NH_4Cl induced similar anomalies, except that polyspermy and exogastrulae did not occur as a result of exposure of eggs to ABS.   Water soluble extracts of Bunker C oil produced anomalies similar to those obtained for ABS, including some polyspermy.   Water soluble extracts of BP 1100X oil dispersant mainly induced reduced blastulae.   Extracts of oil-oil dispersant mixtures produced anomalies similar to those for oil extracts.   The first cell division was inhibited at somewhat lower concentrations of Bunker C extract in the Canadian species, compared with responses in the two Japanese species.   Inhibitory effects of oil-oil dispersant extracts were greater than those obtained for either component tested alone.   In general, minimum effective concentrations of all materials examined were similar in all species considered.   Bioassays using sea urchin eggs are suggested as a possible general technique for judging the toxic effects of marine pollutants.

Acknowledgement

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